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CANELLA, KAREN A	
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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/801,471	Applicant(s) Terstappen et al
	Examiner Karen Canella	Art Unit 1642

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3 months* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☐ Responsive to communication(s) filed on _____.

2a) ☒ This action is FINAL. 2b) ☐ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under ***Ex parte Quayle***, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *1-23, 27-42, 46-50, and 54* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *1-23, 27-42, 46-50, and 54* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

Response to Amendment

1. Claims 24-26, 43-45 and 51-53 have been canceled. Claims 1, 7, 9, 10, 16, 22, 23, 28, 35, 41, 42, 47, 50 and 54 have been amended. Claims 1-23, 27-42, 46-50 and 54 are under consideration.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

3. The objections to the specification and oath/declaration are maintained for reasons of record. Applicant argues that the relevant inquiry in determining 35 U.S.C. 112, is whether the claims set out and circumscribe a particular area with a reasonable degree of precision and particularly such that the metes and bounds of the claimed invention are reasonably clear, and that any consideration should be in light of teachings of the prior art and of the particular application disclosure, as interpreted by one of having ordinary skill in the art. Applicant further states that claims to the labeling of cells in the present invention "albeit not fixed, stabilized, or otherwise", are specifically mentioned in U.S. 6,365,362, and an internal control cell was used in '362. This has been considered but not found persuasive. A claim to priority in a previous application must satisfy the conditions of 112 first paragraph, therefore arguments regarding the metes and bounds and clarity of the claimed invention are irrelevant. The M.P.E.P. states "To comply with the written description requirement of 35 U.S.C. 112, para. 1, or to be entitled to an earlier priority date or filing date under 35 U.S.C. 119, 120, or 365(c), each claim limitation must be expressly, implicitly, or inherently supported in the originally filed disclosure. When an explicit limitation in a claim "is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have understood, at the time the patent application was filed, that the description requires that limitation." Hyatt v. Boone, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998). See also In re Wright, 866 F.2d 422, 425, 9 USPQ2d 1649, 1651 (Fed. Cir. 1989)". As the prior applications to which priority is sought do not describe or require the

stabilized permeablized control cell of the instant invention, one of skill in the art would not conclude that at the time the parent applications were filed that the invention required the stabilized permebilized control cells of the instant invention. If the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or amended claim must be rejected under 35 U.S.C. 112, para. 1, as lacking adequate written description, or in the case of a claim for priority under 35 U.S.C. 119, 120, or 365(c), the claim for priority must be denied.

4. The rejection of claims 1-23, 27-42, 46-50 and 54 under 35 U.S.C. 112, second paragraph, for the recitation of "physically and biologically stable" in claims 1 and 7 is withdrawn in light of applicants argument.

5. The rejection of claims 5, 15 and 32 under 35 U.S.C. 112, second paragraph, for dependence on an object which is variable, is maintained. Applicant argues BODIPY is defined by the specification as the lipophilic type which stain the Golgi apparatus. Hoechst 33342 and BODIPY™ which are trade names. Section 2173.05(u) of the M.P.E.P. states "If the trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of the 35 U.S.C. 112, second paragraph. Ex parte Simpson, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product. In fact, the value of a trademark would be lost to the extent that it became descriptive of a product, rather than used as an identification of a source or origin of a product. Thus, the use of a trademark or trade name in a claim to identify or describe a material or product would not only render a claim indefinite, but would also constitute an improper use of the trademark or trade name. .

6. The rejection of claims 1-23, 27-42, 46-50 and 54 under 35 U.S.C. 112, second paragraph, for the recitation of "redundant labeling...with at least two fluorescent labels having the same spectral properties" in claim 1 and the recitation of "redundant membrane labeling" in claim 7, the recitation of "cell...labeled redundantly" and the recitation of "redundantly labeled"in claims 20, 28 and 37 is maintained for reasons of record. It is unclear what encompasses the claimed redundant labeling as only chemically identical fluorescent moieties would have the same spectral properties. Applicant argues that the dyes have similar but not necessarily identical spectral characteristics. This has been considered but not found persuasive as claim 1 comprises the limitation "at least two fluorescent labels having the same spectral properties". The claim is drawn to dyes which have identical spectral properties, as one of skill in the art would know that "the same spectral properties" is equivalent to "identical spectral properties".

7. The rejection of claims 1-23, 27-42, 46-50 and 54 under 35 U.S.C. 112, second paragraph, for the recitation of "up to at least six months", is withdrawn in light of applicants amendments.

8. The rejection of claim 8 under 35 U.S.C. 112, second paragraph, for lacking proper antecedent basis in claim 7 is withdrawn in light of applicants amendments.

9. The rejection of claim 16 under 35 U.S.C. 112, second paragraph, for the recitation of "detectably labeled membrane" is withdrawn in light of applicants arguments.

10. The rejection of claim 54 under 35 U.S.C. 112, second paragraph, for the recitation of "the method as claimed in claim 47" is withdrawn in light of applicants amendment.

11. Claims 10-21, 28-41, 46--50 and 54 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 10, 16, 28, 35 and 47 have been amended ti recite "stabilized cell, previously permeablized". It is unclear how "previous" permeabilization affects the immediate properties of the claimed cell, or if another alteration to the cell has occurred between the "previous" condition of the cell and the condition of the cell in the immediate time. Amendment of the claims to recite a permeablized, stabilized cell would obviate this rejection.

12. The rejection of claims 22, 23, 41, 42, 49 and 50 under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a control cell further comprising a detectable label bound to an intracellular determinant which is an estrogen receptor or an androgen receptor and a method of using said cell in the detection of rare cells, does not reasonably provide enablement for a control cell further comprising a detectably labeled surface determinant which is an estrogen determinant or an androgen determinant. Is maintained fro reasons of record. Claims 22 and 41 have been amended to replace estrogen receptor with estrogen determinant. Claims 23, 42 and 50 have been amended to replace androgen receptor with androgen determinant. Applicant argues that estrogen or androgen receptor refers to a type of determinant on the cell surface. The art teaches that both the estrogen receptor and the androgen receptor are located in the nucleus (see: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 2001, pages 1606-1607 and pages 1637-1638) within estrogen receptor positive cells, and androgen receptor positive cells, respectively. Neither the specification nor any art of record teaches a surface determinant which is an estrogen or androgen receptor. Further, the specification does not provide any teachings with regard to an estrogen determinant or an androgen determinant which is not an estrogen receptor of an androgen receptor. The specification has not provided any teachings to a separate protein apart from these receptors which could function as an estrogen or

androgen receptor on the cell surface. Accordingly, one of skill in the art would be subject to undue experimentation without reasonable expectation of success in order find an estrogen determinant on the surface of an MCF-7 cell or to find an androgen surface determinant on the surface of a LNCaP cell.

13. Claims 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis (EP 469,766, reference B1 of the IDS filed Sep 25, 2001) as evidenced by Leif et al (US 5,188,935) in view of Terstappen et al (WO 95/13540) and Maples (WO 94/16314) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541) and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84).

Claim 1 is drawn to a process for producing a stabilized cell, said process comprising: the redundant labeling of said control cell with at least two florescent labeled having the same spectral properties; permeabilizing said control cell; contacting said labeled cells with a fixative; removing excess fixative to promote long-term storage, said control cells being biologically stable for a period up to at least six months. Claim 2 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 4 specifies that the label is an antibody immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 5 is drawn to the labeling of cellular components with DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red and lipophilic BODIPY™. Claim 6 specifies the cellular components of nucleic acids, nuclei, lysosomes, Golgi apparatus, mitochondria and endoplasmic reticulum.

Claim 10 is drawn to a stabilized cell, previously permeablized, wherein said control cell is labeled redundantly with at least two fluorescent labels having the same spectral properties and cellular components and antigenic moieties of said control cell have been stabilized for a period up

to at least six months by exposure to fixative. Claim 12 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 14 specifies said control cells immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 15 specifies that the cellular components of said control cells are labeled with DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red and lipophilic BODIPY™.

Claim 28 is drawn in part to a stabilized cell, previously permeablized, comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties , said cells further comprising stabilized cellular components and antigenic moieties , said stabilization being affected by exposure to fixative, wherein said control cells are tumor cells. Claim 29 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 31 specifies said control cells immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 32 specifies that the cellular components of said control cells are labeled with DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red and lipophilic BODIPY™. Claim 33 specifies that the cellular components are nucleic acids, nuclei, lysosomes, Golgi apparatus, mitochondria and endoplasmic reticulum.

Davis teaches a method of making a control cell which is fixed by paraformaldehyde, said excess paraformaldehyde being removed after fixing (column 9, lines 20-30). Davis teaches that cells so fixed retain their ability to be tagged with antibodies conjugated to fluorescent markers, stains such as fluorescein, rhodamine and cyanine dyes and nucleic acid stains (column 5 , line 39 to column 6, line 1). Davis et al teach that said control cells may be derived for leukemias, cancers (column 5, lines 16-20, column 8, lines 4-6) and tumor cell lines (column 8, lines 8-11). Davis teaches that the cells may be dried after fixation but before labeling or conversely may be labeled after fixation but prior to drying. Davis does not specifically teach that the fixed cells or the fixed and dried cells would be biologically stabilized for six months, however, the method of

paraformaldehyde fixation appears to be identical to the claimed method of fixation, therefore it is reasonable to assume that the fixed cells of Davis would be biologically stable for at least six months. Davis does not specifically teach the order of the claimed method steps wherein the cells are labeled before being fixed (column 8, lines 45-49). Leif et al teach that antigen-antibody complexes on leukocytes are preserved after cross-linking with dialdehyde fixing reagents (column 4, lines 4-16). Therefore, it would be reasonable to assume that the control cell of Davis could be labeled before or after fixing with glutaraldehyde or glyoxal. Lief et al do not teach the redundant labeling of the fixed cells, the stability of the fixed cells, nor the labeling of tumor cells or tumor cell lines for use as control cells. Davis does not specifically teach the redundant labeling of the cells with at least two fluorescent labels having the same spectral properties.

Maples et al teach differentially labeled reconstituted control cells. Maples et al teach that the use of said cells represents an improvement in the art over the use of fluorescent beads as said cells have the same size shape and light scatter characteristics of the analyte (page 3, lines 8-31). Thus it can be concluded that the control cells of Maples are labeled with the same fluorescent markers as the analyte to enable the simultaneous analysis of labeled analyte and controls (page 5, lines 9-13). Maples et al do not teach control cells exposed to fixative.

Gross et al teach the redundant labeling of BT-20 cells with antibodies which reacted with the cytokeratins 5, 6, 8 and 18, said antibodies conjugated to the fluorescent moiety PerCP (page 538, second column lines 19-26).

Terstappen et al (WO 95/13540) teach a method of labeling rare cells wherein "one or more monoclonal antibodies, labeled with a first fluorochrome" differentially reacts with all cell populations in the sample (page 5, lines 19-24). Thus it can be concluded that Terstappen et al teach the redundant labeling of analyte cells with a redundant fluorescent moiety. Terstappen et al teach fixed fluorescent cells as control cells (page 6, lines 26-29). Terstappen et al do not teach the method of fixing said control cells or the resulting stability of said control cells.

Any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) teach methods of permeabilizing cells for the labeling of internal antigens within cells.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to redundantly label the control cells taught by Davis with antibodies which immunospecifically bind different target antigens, said antibodies being conjugated to the same fluorescent moiety by a method which allows the internal labeling of antigens. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Gross et al and Terstappen et al (WO 95/13540) on the redundant labeling of rare cells, and the teachings of Maples et al on the improvement in the art affected by the use of control cells labeled with the same fluorescent markers as analyte cells and the teachings of any of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) on the need for permeabilization of cells that will be assayed for internal antigens. One of skill in the art would be motivated to redundantly label control cells which have been previously permeablized because (a) of the teachings of what is well known in the art as exemplified by any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) that cells must be permeablized for labeling of internal antigens, (b) the usefulness of redundantly labeling analyte rare cells has been demonstrated by Gross et al and Terstappen et al, and (c) the teachings of Maples et al directs one of skill in the art to label control cells with the same fluorescent reagents as analyte cells.

14. Claims 1-6, 10, 12-15 and 28-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) as applied to claims 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32, 33 above, and further in view of Gibson et al (Bone Marrow Transplantation, 1988, Vol. 3, pp. 567-576) and Waggoner et al (Human Pathology, 1996, Vol. 27, pp. 494-502) and Haugland (Handbook of Fluorescent Probes and Research Chemicals, 1992, 5th Edition, pp. 235-269). The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) render obvious the embodiments of claims 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32 and 33 for the reasons set forth above.

Claim 3 specifically embodies the method of claim 1 wherein the fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

Claims 13 and 30 specifically embody the control cells of claims 10 and 28, respectively, wherein the fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

Although the combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al render obvious the labeling of the claimed control cells with

fluorescent labels, neither Davis nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al specifically teach the labeling of a cell with the above recited membrane fluorescent labels.

Gibson et al teach the labeling of rare tumor cells with reagents that stain glycolipids (page 567, first column, lines 13-19). Waggoner et al teach the use of diL-C18 as a membrane label to establish the location of the cellular membrane during fluorescence imaging (Table 1, "Membrane location and fluidity"). Dil-C18 is defined as an analog of carboindocyanines as stated on page 25, line 1 of the instant specification.

Haugland teaches the octadecyl ester of fluorescein (page 253, under the heading "Lipophilic Fluorescein Probes"), the octadecyl indocarbocyanines and oxacarbocyanines and analogs thereof, dialkyamnostyrl and octadecyl rhodamine B (pages 260-261), all of which have the property of staining membranes.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the membrane stains taught by Waggoner et al and Haugland for the redundant anti-cytokeratin antibodies as taught by Gross et al in the control cell and method of making said control cell as taught by the combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Gibson et al on the labeling of membrane glycolipids in a method of detecting rare tumor cells in bone marrow aspirates and the teachings of Waggoner et al on the usefulness of membrane probes to establish the location and fluidity of the membrane during fluorescence imaging.

15. Claims 1-10 and 12-16, 18-21, 23 and 28-33 and 35-40, 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Lief et al and Terstappen et al and Maples et al and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994,

Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland as applied to claims 1-6, 10, 12-15 and 28-33 above, and further in view of Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) as evidenced by the abstract of Xing et al (Cancer Research, 1990, Vol. 50, pp. 89-96). The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland render obvious the embodiments of claims 1-6, 10, 12-15 and 28-33 for the reasons set forth above.

Claim 7 is drawn to a process for producing a stabilized control cell comprising: the redundant labeling of said control cell with at least two florescent labels having the same spectral properties; contacting said labeled cells with a fixative; removing excess fixative to promote long-term storage, said control cells being biologically stable for at least six months, wherein said control cell expresses epithelial cell adhesion molecule on its surface and also expresses cytokeratin intra cellularly. Claim 8 specifically embodies the process of claim 7 wherein cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 9 specifically embodies the process of claim 7 wherein the fluorescent label is a membrane label selected from the group consisting of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

Claim 16 is drawn to a stabilized cell, previously permeablized, having determinants in common with rare cells, said stabilized cell comprising a detectably labeled membrane and stabilized cellular components and antigenic moieties due to exposure to fixative, wherein said control cell is a tumor cell expressing EpCAM on its surface and intracellular cytokeratin. Claim 18 specifically embodies the cell of claim 16 wherein said fixative is selected from the group

consisting of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 19 specifically embodies the cell of claim 16 wherein said membrane label is selected from the group consisting of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes. Claim 20 specifically embodies the cell of claim 16 wherein said membrane is redundantly labeled with at least two fluorescent moieties having the same spectral properties. Claim 21 specifically embodies the control cell of claim 16, wherein said cell is a SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin, and Her-2/neu. Claim 23 comprises the control cell of claim 16 wherein said cell is a LNCaP prostate cancer cell further comprising a detectably labeled determinant selected from the group consisting of PSMA, PSA and the androgen determinant.

Claim 35 is drawn to an improved method of detecting an enumerating rare cells in a mixed cell population, the presence of said cells being indicative of severity of a disease state comprising:

(a) obtaining a blood sample suspected of containing said rare cells

(b) preparing an immunomagnetic sample wherein said blood sample is mixed with magnetic particles coupled to a ligand which reacts specifically with a determinant of the rare cells

(c) contacting said immunomagnetic sample with at least one reagent which labels a determinant of said rare cells, and

(d) analyzing the labeled rare cells to determine the presence and number of any rare cells, wherein the greater the number of rare cells present in said sample, the greater the severity of the disease, wherein the improvement comprises the addition of a stabilized cell, previously permeablized, for use as an internal control in said method, said control cell having determinants in common with said rare cells, and wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for at least six months by exposure to fixative.

Claim 36 specifically embodies the method of claim 35 wherein said rare cell is a cancer cell and said disease is cancer. Claim 37 specifically embodies the method of claim 35 wherein said membrane is redundantly labeled with at least two fluorescent labels having the same spectral properties. Claim 38 specifically embodies the method of claim 35 wherein said membrane label is selected rom the group consisting of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes. Claim 39 specifically embodier the method of claim 35 wherein said ligand is an anti-EpCam, said reagent labels intracellular cytokeratin, said EpCam and cytokeratin being present in both rare cell and control cell. Claim 40 specifically embodies the method of claim 35 wherein the control cell is an SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobluin, human milk fat globulin, and HER-2/neu. Claim 42 specifically embodies the method of claim 39 wherein the control cell is an LNCaP prostate cancer cell further comprising a second detectably labeled determinant selected from the group consisting of PSMA, PSA and androgen determinant.

The combination of Davis and Lief et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland render obvious the process of producing a control cell comprising a redundantly labeled membrane, the membrane stains of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and the control cell produced thereby. The combination of Davis and Lief et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland do not teach a method of producing a stabilized control cell expressing epithelial cell adhesion molecule on its surface, a stabilized tumor cell expressing EpCam on its surface, a stabilized SKBR3 breast cell expressing EpCam on its surface and further comprising a detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin and Her-2/neu, or a stabilized LNCaP prostate cancer cell expressing EpCam on its surface and further comprising a detectably labeled determinant selected from the

group consisting of PSMA, PSA and androgen receptor or an immunomagnetic separation of cancer cells from normal cells in a biological specimen wherein a ligand is anti-EpCam in a method of detecting rare cancer cells wherein the detection of said cancer cells is correlated with the severity of the disease and wherein the method is improved by the addition of control cells , said control cells having determinants in common with said rare cells. However, it is noted that the combination of Davis and Lief et al and Terstappen et al and Maples et al and Gross et al do render obvious the control cell comprising the determinant of intracellular cytokeratin as taught by Gross et al (page 538, second column, second paragraph).

Racila et al teach the detection of rare prostate cancer cell and rare breast cancer cells in the blood of patients . Racila et al teach that the greater the number of said rare cells the greater the severity of the disease (page 4592, first column, line 19 to second column, line 2 and Figure 4). Racila et al teach the use of SKBR3 breast cancer cells and LNCaP prostate cancer cells as standards to evaluate the reagents used in the immunocytochemical detection and to determine the sensitivity of the assay (page 4589, second column, under the heading "Cell Lines"). Racila et al teach an immunomagnetic separation of rare prostate cancer cells or rare breast cancer cells from biological specimens, said assay using the ligand anti-EpCam (page 4589, beginning in second column, under the heading of "Sample Preparation for Flow Cytometric Analysis"). Racila et al further teach the labeling of said separated cells with the anti-cytokeratin CAM5.2 antibody (page 4590, first column, lines 6-8), and the further labeling of said separated prostate cells with PSA and the redundant labeling of cytokeratins by means of monoclonal antibodies CK, 5D3, and LP34 (page 4590, first column, lines 24-30). Racila et al do not teach the SKBR3 breast cell further comprising a detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin and Her-2/neu. Racila et al do not teach a method of producing SKBR3 or LNCaP as stabilized control cells comprising redundantly labeled membranes, or the control cells produced thereby, or an improvement to the immunomagnetic separation comprising the addition of said control cells as internal controls.

Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) teach immunomagnetic separation of rare breast cancer cells from biological specimens, said assay using the ligand anti-EpCam (page 573, second column under the heading "Sample preparation") and the further labeling of separated cells with the anti-cytokeratin antibody CAM5.2 (page 574, first column, lines 2-6). Terstappen et al teach a correlation between the number of said rare cells the severity of the disease (Figure 2). Terstappen et al suggest a further assessment of the isolated rare breast cancer cells comprising the detection of mammoglobin, breast mucin and her-2 (page 577, second column , last eight lines). The abstract of Xing et al teaches that human milk fat globulin is a type of mammary mucin that is increased in breast cancer.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the specific cells of SKBR3 or LNCaP as stabilized control cells comprising redundantly labeled membrane for the general tumor cell line control cells taught by the combination of Davis and Lief et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland; and to use said stabilized cells as an improvement to an immunomagnetic separation comprising the addition of said cells as internal controls. It would also be obvious to produce a stabilized SKBR3 control cell further comprising a detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin and Her-2/neu.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Racila et al and Terstappen et al on the use of SKBR3 cells and LNCaP cells as standards in the evaluation of immunocytochemical detection, and the teachings of Maples et al on the improvement afforded to methods for detecting rare cells by the incorporation of biological cells as control cells and the teachings of Terstappen et al (WO 95/13540) on the use of fixed fluorescent cells as control cells in the enumeration of rare cells by flow cytometry (page 6, lines 26-29 and page 5, line 19 to page 6, line 4). One of skill in the art would also be motivated by the teachings of Terstappen et al (International Journal of Oncology,

2000) and Xing et al to further label said control cells for the determinants of milk-fat globulin, mammoglobin and Her-2, as these were suggested by Terstappen (2000) to further study the correlation between said the detection of said determinants in blood and the presence of metastatic breast cancer (page 577, last ten lines).

16. Claims 1-21, 23, 28-40 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al as applied to claims 1-10 and 12-16, 18-21, 23, 28-33 and 35-40, 42 above, and further in view of Young et al (US 5,529,933).

The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al render obvious the embodiments of claims 1-10 and 12-16, 18-21, 23, 28-33 and 35-40, 42 for the reasons set forth above.

Claim 11 specifically embodies the control cell of claim 10, said control cell suspended in a buoyant density medium.

Claim 17 specifically embodies the control cell of claim 16, said control cell suspended in a buoyant density medium.

Claim 34 specifically embodies the stabilized cell of claim 28, said control cell suspended in a buoyant density medium.

Although the combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al render obvious the stabilized fixed cells of the instant invention, neither Davis nor Lief et al nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al nor Gibson et al nor Waggoner et al nor Haugland nor Racila et al nor Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) teach the stabilized, control cells suspended in a buoyant density medium.

Young et al teach stabilized red blood cells fixed with organic aldehydes such as formaldehyde or glutaraldehyde (column 10, lines 11-27) for use as a control in hematological analyses (column 11, lines 61-67). Young et al teach that said fixed red blood cells can be maintained for up to six months in a preferred formulation of a suspension medium (column 14, line 61 to column 15, line 39). Young et al do not teach cells with fluorescent labels for use in detecting rare cells.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to suspend the stabilized control cells as rendered obvious by the combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) in buoyant density medium. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Young et al on the long-term stability of aldehyde fixed cells stabilizing medium.

17. Claims 1-23, 28-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Lief et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al as applied to claims 1-21, 23, 28-40 and 42 above, and further in view of Rao et al (Cancer, 1980, Vol. 46, pp. 2902-2906).

The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al render obvious the embodiments of claims 1-21, 23, 28-40 and 42 for the reasons set forth above.

Claim 22 specifically embodies the control cell of claim 16, wherein said control cell is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen determinant.

Claim 41 specifically embodies the method of claim 39, wherein the control cell is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen determinant.

Please note that the limitation of "surface" determinant has been omitted from the claims for the reasons set forth in the rejection under 35 U.S.C. 112, first paragraph, above.

Although the combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) render obvious the methods and control cells comprising SKBR3 breast cancer cells, neither Davis nor Lief et al nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al nor Gibson et al nor Waggoner et al nor Haugland nor Racila et al nor Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) nor Xing et al nor Young et al teach a control cell which is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor or a method of detecting and enumerating rare cells comprising the use of said control cell.

Rao et al teach a MCF-7 control cell comprising a detectably labeled estrogen receptor 2903, first column, under the heading "Staining with Fluorescein Conjugate" and the use of said control cells in the detection of breast cancer (page 2905, first column, lines 18-23 and page 2904, second column, under the heading "17FE Uptake in Human Breast Cancer). Rao et al suggest the method of flow cytometry as a more precise method of quantitating the fluorescence intensity observed in tumor cells stained for the estrogen receptor. Rao et al do not teach a fixed MCF-7 cell or the use of said fixed control cell in an assay for rare cells.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the MCF-7 control cell for the fixed SKBR3 cell. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Rao et al on the presence of estrogen receptors in both MCF-7 cells and human breast cancer, and the use of MCF-7 cells as control cell in the detection of estrogen receptors in human cancers.

18. Claims 1-23, 28-42 and 47-50, rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Lief et al and Maples et al and Terstappen et al and Gross et al and any of the

abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al as applied to claims 1-23, 28-42 above, and further in view of Terstappen et al (WO 99/41613).

The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al render obvious the embodiments of claims 1-23, 28-42 for the reasons set forth above.

Claim 28 is further drawn to a stabilized cell, previously permeablized, comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties , said cells further comprising stabilized cellular components and antigenic moieties , said stabilization being affected by exposure to fixative, wherein said control cells are selected from the group consisting of tumor cells, bacterially infected cells, viraly infected cells, myocardial cells, circulating endothelial cells, and fetal cells in maternal circulation.

Claim 47 is drawn to an improved kit for the screening of a patient for the presence of circulating tumor cells comprising:

(a) coated magnetic nanoparticles comprising a magnetic core material, a protein base coating material and anti-EpCAM coupled directly or indirectly to said base coating material;

(b) at least one antibody having a binding specificity for a cancer cell determinant;

(c) cell specific dye for excluding sample components for other than said tumor cells; wherein the improvement comprises the addition of a container comprising stabilized cells, previously permeablized, for use in an internal control, said stabilized control cells having determinants in common with said rare cells, wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for up to at least six months by exposure to fixative, said stabilized control cells being suspended in buoyant density medium.

Claim 48 specifically embodies the method of claim 47, wherein said cell is a SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin, and Her-2/neu.

Claim 49 specifically embodies the method of claim 47, wherein said control cell is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor.

Claim 50 specifically embodies the method of claim 47, wherein said cell is a LNCaP prostate cancer cell further comprising a detectably labeled determinant selected from the group consisting of PSMA, PSA and androgen determinant.

The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al renders obvious the stabilized control cells, wherein said cells are SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin, and Her-2/neu; MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor; and LNCaP prostate cancer cell further comprising a detectably labeled surface determinant selected from the group consisting PSA and the use of said control cells in an method

for the detection and enumeration of rare tumor cells using an immunomagnetic separation for the reasons set forth above.

Davis further teaches a control slide kit for image analysis comprising cells which have been fixed with paraformaldehyde, reduced with a Schiff's base after fixation and then dried onto a slide or into a capillary tube with a protein, membrane stabilizing compounds (column 5, lines 23-28). Davis does not specifically teach tumor cells having the Ep-CAM determinant, or the enumeration and isolation of rare cells in circulation.

Maples et al teach differentially labeled reconstituted control cells. Maples et al teach that the use of said cells represents an improvement in the art over the use of fluorescent beads as said cells have the same size shape and light scatter characteristics of the analyte (page 3, lines 8-31). Thus it can be concluded that the control cells of Maples are labeled with the same fluorescent markers as the analyte to enable the simultaneous analysis of labeled analyte and controls (page 5, lines 9-13). Maples et al do not teach control cells exposed to fixative.

Terstappen et al (WO 95/13540) teach fixed fluorescent cells as control cells (page 6, lines 26-29). Terstappen et al do not teach the method of fixing said control cells or the resulting stability of said control cells.

Young et al teach stabilized red blood cells fixed with organic aldehydes such as formaldehyde or glutaraldehyde (column 10, lines 11-27) for use as a control in hematological analyses (column 11, lines 61-67). Young et al teach that said fixed red blood cells can be maintained for up to six months in a preferred formulation of a suspension medium (column 14, line 61 to column 15, line 39). Young et al do not teach cells with fluorescent labels for use in detecting rare cells. However, Lief et al teach the maintenance of antibody-cell complexes through the aldehyde fixing process.

Terstappen et al (WO 99/41613) teach a kit for the screening of a patient for the presence of circulating tumor cells comprising:

(a) coated magnetic nanoparticles comprising a magnetic core material, a protein base coating material and anti-EpCAM coupled directly or indirectly to said base coating material;

(b) at least one antibody having a binding specificity for a cancer cell determinant;

(c) cell specific dye for excluding sample components for other than said tumor cells claims 48-62 of WO 99/41613).

Terstappen et al (WO 99/41613) further teach the claimed immunomagnetic method for the detection of circulating rare cells selected from the group consisting of endothelial cells, fetal cells in circulation, bacterial cells, myocardial cells and viraly infected cells (claim 6).

Neither Davis nor Lief et al nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al nor Gibson et al nor Waggoner et al nor Haugland nor Racila et al nor Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) nor Xing et al nor Young et al and Rao et al teach an improved kit for the screening of a patient for the presence of circulating tumor cells comprising: a container comprising stabilized cells for use in an internal control, said stabilized control cells having determinants in common with said rare cells, wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for up to at least six months by exposure to fixative, said stabilized control cells being suspended in buoyant density medium, wherein the control cells are SKBR3, MCF-7 or LNCaP.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to

(a) use the control cells rendered obvious by the combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al as an improvement to the kit for detecting circulating rare cells in patient; and

(b) to make stabilized control cells comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties , said cells further comprising stabilized cellular components and antigenic moieties , said stabilization being affected by exposure to fixative, wherein said control cells are, in addition to the tumor cells rendered obvious by the combination of the prior art references stated in this section, are selected from the group consisting bacterially infected cells, viraly infected cells, myocardial cells, circulating endothelial cells, and fetal cells in maternal circulation.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Davis on a kit comprising paraformaldehyde fixed cells for use as a control slide in image analysis, and the teachings of Terstappen et al (WO 95/13450) on the use of fixed fluorescent cells as control cells, and the teachings of Maples et al on the improvements afforded by the use of biological cells as control cells (versus fluorescent beads) and the teachings of Young et al on the stability of a cell fixed with aldehydes and stored in a suspension medium; and the teachings of Terstappen et al (WO 99/41613) on an immunomagnetic method for the detection of circulating rare cells selected from the group consisting of endothelial cells, fetal cells in circulation, bacterial cells, myocardial cells and viraly infected cells .

19. Claims 1-23, 27-42 and 46-50 and 54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Terstappen et al and Lief et al and Maples et al and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578)and Xing et al and Young et al and Rao et al and Terstappen et al (WO 99/41613) as applied to claims 1-23,

28-42 and 47-50 above, and further in view Shih (Journal of Pathology, 1999, Vol. 189, pp. 4-11) and Shih et al (Cancer Research, 1994, Vol. 54, pp. 2514-2520) and the abstract of Silverstein et al (Journal of Biological Chemistry, 1992, Vol. 267, pp. 16607-16612)

The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and any of the abstracts of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138, pp. 143-153) or Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) or Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al and Terstappen et al (WO 99/41613) render obvious the embodiments of claims 1-23, 28-42 and 47-50 for the reasons set forth above.

Claim 27 specifically embodies the control cell of claim 16, wherein said control cell is a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD146 molecule.

Claim 46 specifically embodies the method of claim 39, wherein the control cell is a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD146 molecule.

Claim 54 specifically embodies the kit of claim 47, wherein the control cell is a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD146 molecule.

The combination of Davis and Lief et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al and Terstappen et al (WO 99/41613) render obvious the claimed control cells, methods and kits of the instant claims 27, 46 and 54 with the exception of the C32 melanoma cancer cell detectably labeled on the CD146 molecule.

Terstappen et al (WO 99/41613) teach that melanoma can be detected using the immunomagnetic compositions, kits and methods disclosed therein (page 75, lines 8-10 and 17). Terstappen et al do not teach C32 control cells comprising a detectable label on the CD146 molecule.

The Shih (1999) teaches that the detection of CD146 is useful in the diagnosis of melanoma (abstract). Shih further teaches that a synonym for the CD146 antigen is the A32 antigen (page 4, second column, lines 1-6).

Shih et al (1994) teach that the A32 antigen is expresed on the cell surface of most melanomas and on melanoma cell lines (abstract and Figure 7 on page 2519).

The abstract of Silverstein et al teaches that C32 cells are wild-type melanoma cells. It would be reasonable to conclude that C32 cells expressed the CD146/A32 antigen.

Neither the abstract of Shih or Silverstein et al teach fixed stabilized C32 cells comprising a detectably labeled CD146 molecule.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute C32 cells having a detectably labeled cell surface determinant which is a CD146 molecule for the general tumor cells taught by Davis. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of the abstract of Silverstein on C32 cells being representative of melanoma and the teachings of Shih et al on the CD146/A32 molecule as being diagnostic for melanoma and the presence of the CD146/A32 antigen on melanoma cell lines.

20. Applicant has amended claims 1, 10, 16, 28, 35 and 47 to incorporate the limitation of a permeablized cell in order to obviate the prior art rejections. It is noted that the permeabilizing solution used by the instant specification was known in the art as it is identified on page 9, paragraph 0091 as Immunicon Part No. 6025. Further, it is well known in the art as exemplified by the references of Labalette-Houache et al (Journal of Immunological Methods, 1991, Vol. 138,

pp. 143-153), Decaestecker et al (Journal of Immunological Methods, 1992, Vol. 154, pp. 11-20) and Yang et al (Journal of Immunological Methods, 1994, Vol. 172, pp. 77-84) teach methods for permeabilizing cells for the labeling of internal antigens within said cells versus external antigens on the surface of the cell membrane. It would have been obvious to permeabilize the stabilized cells for the purpose of labeling internal antigens.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

21. All other rejections and objections as set forth in Paper No. 3 are withdrawn.

Conclusion

22. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however,

will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Karen A. Canella, Ph.D.
Patent Examiner, Group 1642
February 27, 2003

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